3200 IDS Center
80 South Eighth Street
Minneapolis, Minnesota
55402-2215 USA
TEL 612.332.5300
FAX 612.332.9081
www.merchant-gould.com
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FROM: Katherine M. Kowalchyk

OUR REF: 8076.231USW1
TELEPHONE: 612.371.5311

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DECLARATION

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SYSTEM OF CHAIN POLYMERASE AMPLIFICATION OF TARGET NUCLEIC ACID SEQUENCES

The present invention concerns the field of genetics.

More precisely, the present invention concerns a system for amplifying target nucleic acid sequences by the polymerase chain reaction and a device for carrying out this procedure.

The present invention finds numerous applications, notably in the area of diagnostic procedures based on the detection of nucleic acids.

Diagnostic procedures based on nucleic acids almost systematically involve a molecular hybridization reaction between a target nucleic acid sequence and one or more nucleic acid sequences complementary to that target sequence, present a number of variations, such as techniques known to the skilled person as "transfer techniques" (blot, dot blot, Southern blot, Restriction Fragment Length Polymorphism, etc.), or such as miniaturised systems on which the complementary sequences of the target sequences are previously fixed (microarrays). Within the context of such techniques, complementary nucleic acid sequences are generally termed probes. A further variation, which can in itself constitute the basis of a diagnostic procedure or may simply be a supplementary step in one of the techniques mentioned above (in particular to increase the concentration of the target sequence and thus, the sensitivity of the diagnosis), consists of amplifying by Polymerase Chain Reaction (PCR). Within the context of that technique, complementary nucleic acid sequences of target sequences, termed primers, are used to amplify those target sequences.

PCR reactions involve repeated cycles, generally 20 to 50 in number, and each is composed of three successive phases, namely: denaturation, hybridization, elongation. The first phase corresponds to transforming

double-stranded nucleic acids into single-stranded nucleic acids; the second phase is molecular hybridization between the target sequence and the complementary primers for said sequence, and the third phase corresponds to elongation of the complementary primers hybridized to the target sequence, using a DNA polymerase. Those phases are carried out at specific temperatures: generally, 94°C for denaturation, 72°C for elongation, and between 30°C and 65°C for hybridization, depending on the hybridization temperature (Tm) of the primers used. Generally, the PCR employs reaction volumes of 2 µl to 50 µl and is carried out in tubes, microtubes, capillaries or systems known in the art as "microplates" (integral assemblies of microtubes). Each batch of tubes or equivalent containers must thus be successively heated to the three temperatures, and for the desired number of cycles.

Using tubes or similar systems obliges the operator to carry out many manipulations to prepare as many tubes and solutions (known in the art as mix PCR) as there are target sequences to be amplified, even when using a single sample of nucleic acids, with the exception of multiplex amplification procedures, which amplify a plurality of target sequences simultaneously in the same container, either using low specificity primers that can hybridize with a plurality of target sequences, such as RAPD – random amplified polymorphism DNA, or using specific primers in larger numbers, where each pair of primers used amplifies a single target sequence. Multiplex amplifications correspond to particular cases and are not in routine use. Further, they do not guarantee freedom from interactions of one amplification reaction with another, and because of possible hybridizations between primers, can only be very limited in the number of target sequences amplified per container.

Those different manipulations cause a number of disadvantages.

Firstly, they are time consuming. Secondly, they are not risk-free as regards possible contamination from one tube to another or from the external environment (dust, bacteria or other contaminants that may contain nucleic acid molecules). Further, homogeneity of volume and reagent concentration from one tube to another is not guaranteed. Finally, the volumes are necessarily manipulated manually and are generally greater than 1 µl, which affects the costs of carrying out PCR as the reagents employed are expensive.

The use of devices designed for at least partial automation of such manipulations can overcome some of those disadvantages. However, those instruments are relatively expensive and their use is, therefore, only economically justified when carrying out many PCR amplifications, for example for genome sequencing.

Thus, there is a need for a Polymerase Chain Reaction amplification system, which does not have the disadvantages of the prior art mentioned above.

The object of the present invention is to provide such a device that can considerably reduce the number of manipulations required to carry out a PCR on a plurality of target sequences and as a result, to reduce the time necessary for this operation.

Another object of the present invention also provides such a device that minimizes the risk of contamination between containers.

Another object of the present invention further provides such a device that reduces the volumes of reagents used, thereby reducing the costs involved.

Still another object of the present invention provides such a device that optimizes homogeneous volume distribution and concentration of the reagents required for PCR in the containers.

These different objects are achieved by the present invention which concerns a device for amplification by polymerase chain reaction of target nucleotide sequences, characterized in that it comprises:

- at least one cartridge having a plurality of reaction chambers; and,
- at least one heating plate having three distinct zones that can be heated to three different temperatures corresponding to the three cycle phases of a Polymerase Chain Reaction (PCR).

Such a device of the invention is less complex than prior art systems, in that the three temperatures necessary for the cycles of PCR are provided by three distinct constant temperature zones, and not by a block the temperature of which is varied.

Different variations in the device described above can be envisaged. In a preferred variation of the invention, the system comprises the following features:

- at least one cartridge presenting at least one reservoir intended to receive a fluid composed of a sample of nucleic acids to be analyzed and the reagents required for a chain amplification reaction, with the exception of primers; a plurality of reaction chambers in which are prepartitioned the specific primes of the target sequence to be amplified and channels connecting the reservoir to the reaction chambers;
- at least one heating plate having three distinct zones that can be heated to three different temperatures corresponding to the three phases of the cycles of PCR;
- means for relatively displacing the plate and the heating plate.

In a preferred variation, it is possible to distribute, from a reservoir, a fluid containing a sample of nucleic acids to be analysed and the reagents

necessary for PCR in a plurality of reaction chambers containing specific primers for the target nucleic acid sequences to be amplified, and to cause the amplification process by continuously subjecting the contents of the chambers to three different temperatures in succession (namely those required for denaturation, hybridization and elongation) a plurality of times by means of a relative movement between the cartridge including said reaction chambers and said heating plate having three distinct zones that can be heated to three different temperatures.

The device described above has the advantage of simultaneously filling all the reaction chambers, which reduces the preparation time and the risks of contamination from one chamber to another. This system also has the advantage of being capable of miniaturization which means that smaller volumes of reagents can be used than was customary with the prior art. Finally, it can also be noted that, because of the specific heating plate that is recommended, the invention can accelerate the PCR cycles since the different phases (denaturation, hybridization, elongation) are not carried out by varying the temperature of the heating plate or the atmosphere as in the prior art, the relative movement between the cartridge and the plate enabling the contents of each of the reaction chambers to be rapidly and successively subjected to the three distinct temperatures of these phases.

It should be noted that a cartridge of the invention can have a multitude of shapes. However, in a preferred variation of the invention, this cartridge is circular in shape, the reservoir then being substantially at the centre of the cartridge, the reaction chambers being distributed in a circle around the reservoir, and the channels connecting the reservoir to the chambers being essentially radial. Such an architecture can optimize filling the reaction chambers from the central reservoir.

Preferably again, said reaction chambers are provided at the relative periphery of said chamber. It is possible to optimize the number of reaction

chambers that can be provided in the cartridge and filled from the central reservoir.

In a variation of the invention, such a cartridge comprises as many channels as there are reaction chambers. However, in some embodiments, sections of the channels may be common to more than one reaction chamber.

When, as described above, the cartridge of the device is circular, distinct heating zones in the heating plate are divided into three sections of a disk. Each portion can be heated to a distinct temperature to successively heat to three distinct temperature the contents of said reaction chambers, by means of relative displacement between the cartridge and the heating plate.

Regarding the displacement means, it should be noted that in a preferred embodiment of the invention, the heating plate is fixed and cartridge is moved by the displacement means.

However, in other embodiments, the cartridge may be fixed and the heating plate may be moved by the displacement means.

In a particularly preferred embodiment of the invention, in which the cartridge is circular, the displacement means rotate said cartridge and/or said heating plate.

Means for supplying the fluid present in said reservoir to said reaction chambers can be produced in different forms. In one preferred variation of the invention, the fluid contained in the reservoir is distributed to the reaction chambers under pressure to allow the chambers to fill in a uniform manner. In this case, the supply means preferably include a piston device with a rate of penetration into the reservoir that is calculated to encourage correct filling of the reaction chambers.

A variety of capacities can be employed for the reservoir intended to receive the nucleic acid sample and the reagents necessary for PCR can vary with the embodiments. Preferably the reservoirs have a capacity in the range of about 0.1 ml to about 1 ml.

The cartridge preferably comprises about 20 to about 500 reaction chambers.

The volume of these chambers depends on the embodiments. Advantageously, the volume of these chambers is in the range of about 1 µl to 10 µl.

The diameter of the channels is preferably selected so as to be sufficiently small not to allow distribution of the fluid present in the reservoir to the reaction chambers under gravity and to prevent non reproducible filling of the chambers. This diameter is preferably about 0.2 mm or less. Regarding this diameter, it should be noted that the cross section of the channels is preferably circular, but it may be any other shape, in particular polygonal, and the "diameter" of the channels will designate the largest cross sectional dimension.

The depth of the reaction chambers (compared with the channels) can also vary as a function of the embodiments of the invention. In a preferred variation, the depth of these chambers is in the range of about 0.5 mm to 1.5 mm.

As indicated above, one advantage of the present invention is that the device can readily be miniaturized. Thus, advantageously, the cartridge has a diameter in the range of about 1 to 10 cm.

It should also be noted that the thickness of the cartridge depends on several factors, in particular on its constituent material. In practice, this

cartridge is preferably constituted by a plastic material and has a thickness between 0.5 to 5 mm.

A conductive element may be provided between the cartridge and the heating plate. However, in a preferred variation of the invention, said cartridge is in direct contact with said heating plate. In this case, said heating plate is advantageously provided with a coating encouraging displacement between said cartridge and said plate. Such a coating can, for example, be constituted by Teflon (registered trade mark).

In order to facilitate thermal exchanges between the contents of the reaction chambers and the heating plate, the "floor" thereof is preferably as thin as possible. Its thickness depends on the material used to produce the cartridge. Preferably, it is in the range of 0.2 mm.

The reaction chambers for the cartridges of the invention are closed, for example, by a transparent upper wall in transparent plastic, to allow the air they contain to escape when they are filled with the fluid from the reservoir.

In a preferred embodiment of the invention, the reaction chambers each comprise two primers specific to a target sequence to be amplified and optionally at least one labelled probe specific to the target sequence.

As indicated above, the heating plate of the system can have three zones that can be heated to distinct three temperatures. Preferably, this plate is constituted by three distinct independent thermal blocks (thermoblocks) connected to means for programming their temperature. One of these thermoblocks is heated to the denaturing temperature, the second to the hybridization temperature, and the third to the elongation temperature. The use of such constant temperature thermoblocks simplifies production of the heating plate.

The means for relative displacement of the cartridge with respect to the plate can be produced in many forms. In one preferred embodiment, the cartridge has at least one lug and the displacement means include at least one axle co-operating with said lug to move said cartridge in a rotary motion.

Advantageously, the device of the invention also comprises optical fluorescence excitation / measuring means provided above said cartridge. In a preferred variation of the invention, these means will constitute a single fixed system. One advantage of a preferred variation of the invention in which the cartridge is circular and moves in rotation is that it can bring each reaction chamber to a position beneath the optical system in succession, thus reducing its complexity.

The invention also concerns any process for nucleic acid amplification by PCR using a device as described above, characterized in that it comprises the following steps:

- at least partially filling a reservoir with a fluid containing a sample of nucleic acids to be analyzed and all that is required for an amplification reaction, with the exception of primers, and optionally, a fluorescent intercalating agent;

- distributing said fluid in the reaction chambers provided in the cartridge, in which the primers and optionally one or more labelled probes specific for the target nucleic acid sequence is/are distributed;

- employing means for relative displacement between the cartridge and the heating plate to successively bring the contents of each chamber to the temperatures defined by the three zones of said heating plate, as many times as is desired.

The mode of relative displacement between the heating plate and the cartridge can vary depending on the embodiment. It may involve

displacement at a continuous rate or intermittently. The displacement rate may be constant, or it may change with time.

The invention and its various advantages will be better understood from the following description of some non limiting embodiments, illustrated in the Figures in which:

- Figure 1 shows a side view of the device of the present invention;
- Figure 2 shows a top view of the heating plate;
- Figure 3 shows a perspective view of a cartridge provided with reaction chambers and part of the displacement means;
- Figure 4 shows a cross section of the cartridge along the line AA.

The device for detecting and quantifying target nucleic acid sequences shown in Figure 1 comprises a circular cartridge (1) of plastic material 2 mm thick with a diameter of 5 cm. This cartridge (1) is provided with a central reservoir (2) and will be described in more detail with reference to Figures 3 and 4. The capacity of the reservoir is 400 µl. Its floor is flat but it should be noted that in other embodiments, it may be domed to facilitate the passage of fluid into the chambers without the formation of air bubbles, in particular at the end of distribution when the reservoir is almost empty.

The device also comprises a heating plate (3) in direct contact with the lower surface of cartridge (1) and means (4) for displacing the cartridge (1) with respect to the heating plate (3). These displacement means include a micromotor (7) connected to two axles (5) that co-operate with two lugs (6) on the cartridge (1) to cause it to move in a rotary motion on the heating plate (3), the latter remaining stationary.

The device described also comprises a piston (8) for co-operating with said reservoir (2) and a fixed optical fluorescence excitation / measuring device

(9) (emitting source to excite at a given programmable wavelength and a receiver for the emitted fluorescence) located above the cartridge (1) and the heating plate (3).

As can be seen in Figure 2, the heating plate (3) is constituted by three metallic blocks (3a, 3b, 3c) (hereinafter termed thermoblocks) in the form of sections of disks. It should be noted here that in this embodiment, these thermoblocks are substantially the same size, but in other embodiments they may be of a different size, "size" meaning its angular extent viewed from above. Each thermoblock (3a, 3b, 3c) is designed to be able to be brought to a constant, programmable temperature corresponding to one of the phases (denaturation, hybridization or elongation) of the amplification cycles (PCR), i.e., in general, respectively 94°C for denaturation, 72°C for elongation and between 30 and 60°C for hybridization depending on the Tm (hybridization temperature) of the primers used. The temperatures of the thermoblocks can be controlled using any means known in the art.

Referring to Figure 3, cartridge (1) is provided with a central reservoir (2) with a capacity of 400 µl connected to 36 reaction chambers (10) by the same number of channels (11) uniformly distributed over the entire periphery of the cartridge (Figure 3 only shows a few of the channels and chambers). These reaction chambers (10) are provided with vents (12) opening at the edge of cartridge (1). In the present embodiment, the channel diameter is 0.2 mm and the volume of the reaction chambers is 2.5 microlitres. In other embodiments, this diameter and volume may, of course, be different.

As already described, this cartridge (1) is also provided with two lugs (6) each pierced by an orifice to allow the passage of an axle (5) connected to the micromotor (7).

In Figure 4, the reaction chambers have a depth of 1 mm. Their floor is about 0.2 mm thick. This is sufficiently thin to facilitate good thermal exchange between the chambers (10) and the thermoblocks (3a, 3b, 3c). The upper portions of reaction chambers (10) are closed by a transparent wall (13), also forming the wall of reservoir (2).

The illustrated device is used as follows.

Central reservoir (2) is intended to receive the nucleic acid sample to be analysed as well as all the components required for the amplification reaction, and optionally a fluorescent nucleic acid reporter (this ensemble is termed the fluid), with the exception of primers pre-deposited in each peripheral reaction chamber (10).

In the present embodiment, the operator places 90 µl (i.e., 36 times 2.5 µl) of fluid, including 75 ng of nucleic acids, in the central reservoir. The concentrations of the reagents in said fluid are as follows:

dNTPs: 200 µM

Taq buffer: 1 x

$MgCl_2$: 1.5 mM

Taq: 4 U

SybrGreen (registered trade mark): 1 x

H_2O: qsp

Each chamber (10), apart from the few with negative controls, contains two specific primers for a target sequence to be amplified, and optionally one or more labelled probes, allowing specific subsequent fluorescence measurement. In the present embodiment, 10 ng of each primer has been deposited in each chamber apart form those acting as the negative control.

After partially filling reservoir (2) with the fluid, wherein the volume is equal to the sum of the volumes of the chambers (the volume of one chamber is defined as being the product of the surface area of the "floor" multiplied by its depth), piston (8) is actuated to distribute the fluid in the plurality of reaction chambers (10). This piston can increase the pressure in reservoir (2) and allows the passage of fluid into the channels towards the chambers. The rate of displacement of the piston in the reservoir is about 1 mm per second and said displacement is halted at a level that depends on the volume of fluid to be distributed to the chambers.

The small diameter of channels (11) prevents fluid diffusion from reservoir (2) to channels (11) and chambers (10) under gravity (on this scale, processes that can usually be ignored, such as capillary forces, become important. and in this case are sufficient to retain the fluid in the reservoir). Because of vents (12), the air present in the chambers (10) is evacuated, which ensures that they are filled.

Thermoblocks (3a, 3b, 3c) are heated to the three temperatures corresponding to the three temperatures of the PCR phases (or to slightly higher temperatures to compensate for any heat losses between the heating plate (3) and cartridge (1)) and the displacement means (4) are actuated to move the cartridge (1) to cause each reaction chamber to pass successively, and for the desired number of times, over the three thermoblocks.

More precisely, block (3a) is heated to the temperature corresponding to the denaturation phase (94°C), thermoblock (3b) is heated to the temperature corresponding to hybridization (36°C) and thermoblock (3c) is heated to the temperature corresponding to the elongation phase (72°C).

In the present embodiment, micromotor (7) for displacement means (4) is designed to cause rotation by 10 degrees every 2.5 seconds (i.e., one PCR

cycle in 1.5 minutes). However, in other embodiments, this movement may be at a different rate and may be continuous instead of being intermittent.

It should be noted that the optical device (9) is provided above the corresponding block 3c heated to a temperature corresponding to the elongation temperature, and more particularly in a location that corresponds to the end of the elongation phase.

The device enables a large number of reaction chambers to be filled rapidly and in a reproducible manner and allows the contents of the chambers to undergo PCR; it also allows fluorescence measurements to be made for each PCR cycle.

The embodiment described above is not intended to limit the scope of the invention. Thus, a number of modifications can be made thereto without departing from the scope of the invention.

CLAIMS

1. A device for carrying out a polymerase chain reaction of target nucleotide sequences, characterized in that it comprises:
   - at least one cartridge (1) having a plurality of reaction chambers (10); and
   - at least one heating plate (3) having three distinct zones that can be heated to three different temperatures, corresponding to three phases of cycles of amplification by polymerase chain reaction.

2. A device according to claim 1, characterized in that it comprises:
   - at least one cartridge (1) having at least one reservoir (2) is intended to receive a fluid composed of a sample of DNAs to be analyzed and the reagents required for a polymerase chain amplification reaction, with the exception of primers;
   - a plurality of reaction chambers (10) in which the specific primers of the target sequence are predistributed and the channels (11) are connected to reaction chambers;
   - at least one heating plate (3) present in three distinct zones that can be heated to three distinct temperatures corresponding to the polymerase chain reaction amplification cycles.

3. Device according to claim 2, characterized in that the cartridge (1) is circular, the reservoir (2) is noticeably predisposed at the center of the cartridge in which the reaction chambers (10) are redistributed in circles around the reservoir (2) and the channels (11).

4. Device according to claim 3, characterized in that said reaction chambers (10) are predisposed at the outside of said cartridge.

5. Device according to any one of claims 2 to 4 characterized in that it comprises as many channels (11) as reaction chambers (12).

6. Device according to any one of claims 3 to 5, characterized in that the distinct heating zones of the heating plate (3) are distributed in three portions of the disk.

7. Device according to any one of claims 2 to 6, characterized in that the heating plate (3) is fixed and the cartridge (1) is moved by means of displacement means.

8. Device according to anyone of claims 2 to 7, characterized in that the cartridge is fixed and the heating plate is moved by means of displacement means.

9. Device according to anyone of claims 2 to 8, characterized in that the means of displacement (4) causes rotation of the cartridge (1) and/or the heating plate (3).

10. Device according to any one of claims 2 to 9 characterized in that it further comprises means for supplying under pressure fluid present in the reservoir to the reaction chambers.

11. Device according to claim 10, characterized in that the supply means include a piston device (7).

12. Device according to any one of claims 2 to 11, characterized in that the reservoir (2) has a size between about 0.1 ml and about 1 ml.

13. Device according to any one of claims 2 to 12, characterized in that is comprises between about 20 and about 500 reaction chambers (10).

14. Device according to any one of claims 2 to 13, characterized in that the reaction chambers (10) have a volume comprised between about 1 µl and 10 µl.

15. Device according to any one of claims 2 to 14, characterized in that the channels (11) have a diameter inferior or equal to about 0.2 mm.

16. Device according to any one of claims 2 to 15, characterized in that the reaction chambers (10) have a depth comprised between about 0.5 mm and 1.5 mm.

17. Device according to any one of claims 2 to 16, characterized in that the cartridge (1) has a diameter comprised between about 1 and 10 cm.

18. Device according to any one of claims 2 to 17, characterized in that the cartridge (1) has a thickness comprised between 0.5 and 5 mm.

19. Device according to any one of claims 2 to 18, characterized in that the cartridge (1) is plastic.

20. Device according to any one of claims 2 to 19, characterized in that the cartridge (1) is in direct contact with the heating plate (3).

21. Device according to any one of claims 2 to 20, characterized in that the heating plate is supplied with a coating encouraging displacement between said cartridge and said heating plate.

22. Device according to any one of claims 2 to 21, characterized in that the floor of the reaction chambers has a thickness of about 0.2 mm.

23. Device according to any one of claims 2 to 22, characterized in that said reaction chambers (10) are disposed with vents (12).

24. Device according to any one of claims 2 to 23, characterized in that the reaction chambers (10) are closed by an upper transparent cavity.

25. Device according to any one of claims 1 to 24, characterized in that the heating plate (3) includes three distinct thermoblocks (3a, 3b, 3c) connected to means for programming their temperature.

26. Device according to any one of claims 1 to 25, characterized in that the cartridge (1) has a lug (6) and the means of displacement (4) include at least one driver (5) cooperating with said lug (6) to cause the cartridge (1) to move in a rotary motion.

27. Device according to any one of claims 2 to 26, characterized in that the reaction chambers (10) each contain two primers specific for the target sequence to be amplified and optionally at least one labeled probe specific for the target sequence.

28. Device according to any one of claims 1 to 27, characterized in that it comprises optical means (9) for fluorescence excitation/ measurement disposed above the cartridge.

29. A method for amplifying by polymerase chain reaction using a device according to any ones of claims 2 to 28, characterized in that it comprises the following steps:

- at least partially filling a reservoir with a fluid containing a sample of nucleic acids to be analyzed and the components required for

carrying out an amplification reaction, with the exception of primers, and optionally, a fluorescent nucleic acid reporter;

- distributing said fluid to the reaction chambers of the cartridge, in which are located the primers, and optionally one or more labeled probes specific for the target nucleotide sequence;
- employing means for relative displacement between the cartridge and the heating plate to successively bring the contents of each reaction chamber to three temperatures defined by three zones of said heating plate.

30. Heating plate (3) for amplifying by polymerase chain reaction of target nucleotide sequences characterized in that it has three distinct zones and can carry three different temperatures corresponding to the three phases of the cycles of amplification by Polymerase Chain Reaction (PCR).

31. Heating plate (3) according to claim 30, characterized in that it has three distinct thermoblocks (3a, 3b, 3c) connected to means for programming their temperature.